# FINAL REPORT

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ULTRA-HIGH ERUCIC ACID BRASSICA BIOTECHNOLOGY
INITIATIVE FOR SASKATCHEWAN

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TECHNICAL AND COMMERCIAL ASSESSMENT OF CANADA - SASKATCHEWAN AGRI-FOOD INNOVATION AGREEMENT PROJECT (APPLICATION NUMBER CODE 96000 414)

# ULTRA-HIGH ERUCIC ACID *BRASSICA* BIOTECHNOLOGY INITIATIVE FOR SASKATCHEWAN

## ANALYSIS OF INTELLECTUAL PROPERTY ISSUES

PREPARED FOR THE CANADA-SASKATCHEWAN AGRI-FOOD INNOVATION FUND.

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The information presented in the present report represents opinion derived as a result of the analysis of publicly available documents and third party information. Although it is believed that the report accurately reflects the current state of the intellectual property related to high erucic acid *Brassica* crops, some information is not available for a variety of reasons. Therefore some of the opinions expressed in this document may not reflect confidential information which may or may not become publicly available in the future. The *FAAR Biotechnology Group* believes however, that this report accurately reflects the current situation and future prospects but does not warrant that any business, intellectual property or research decision taken by Agriculture and Agri-Food Canada in consideration of this information is justified.

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## INTRODUCTION

The technology described in the AFIF proposal entitled: "***Ultra-High Erucic Acid Brassica Biotechnology Initiative for Saskatchewan***" submitted by Mac Kenzie and Taylor (referred to as the high erucic acid technology) details the experimental protocols required for development and commercialization of a super high ( greater than 66%), erucic acid *Brassica* oilseed crops.

The basis of this technology is the genetic transformation of plant cells with an acyltransferase gene from yeast (SLC-1) which leads to an overall increase in oil content and in erucic acid storing varieties, the potential for the accumulation of very high levels of erucic acid. The proposal further describes the use of additional genes related to fatty acid metabolism, specifically the fatty acid elongase gene (FAE 1) which is proposed to drive the biosynthesis of very long chain fatty acids and the delta 5 desaturase (DE5S) gene which can be used to produce a specialty dienoic fatty acid.

The primary enabling aspect of the technology involves the discovery that the yeast SLC-1 gene, when expressed in plant cells, behaves somewhat differently that the corresponding plant enzyme and is capable of inserting long chain fatty acids in the *sn-2* position of the glycerol backbone of stored triglyceride fatty acids. The yeast gene appears to have a novel preference for the use of very long chain fatty acids whereas the corresponding plant activity does not exhibit this marked preference. It has been postulated that one of the limitations for producing very high levels of erucic acid in conventional erucic acid cultivars is the inability to effectively add erucic acid to the *sn-2* position of the glycerol backbone. Hence the use of the yeast SLC-1 gene overcomes this limitation and allows for the accumulation of very high levels of erucic acid in the form of tri-erucin. The acyltransferase gene is specified as the "SLC" technology in the present report. The yeast SLC-1 gene was discovered by researchers at the University of Kentucky. The University has filed a patent on the gene which is actually an altered form of a normal yeast gene. The use of the gene is controlled by the University, however the NRC has an entered agreement that allows use of the gene in *Brassicas* and other crops. Accordingly, there are no intellectual property barriers to the use of the gene itself, however there concerns relating to component technologies and transformation methods.

The other aspect of the proposed research involves the use of two additional genes, the FAE 1 and DE5S genes which will be obtained from published sources (FAE 1) or cloned from meadowfoam (*Limnanthes douglasii*). It is postulated that addition of FAE 1 gene to SLC-1 plants will drive the increased accumulation of erucic acid.

The addition of the DE5S gene will permit the production of a 22:2 (desaturated at the 5 and 13 position) dienoic fatty acid which has particular industrial utility. Accordingly the intellectual property related to these genes is also of interest in

evaluating freedom to operate issues. Therefore information on the patents related to high erucic acid, desaturase and elongase genes and *Brassica* transformation is also provided.

It should be noted that in view of the current status of many plant biotechnology inventions, it is essentially impossible to give an incontestable opinion on issues related to intellectual property and freedom to operate, particularly if the patents are still pending applications. It is more equivocal to consider the impact of issued US patents since claimed subject matter is usually well-defined in the issued claims. However, it should be noted that issued US patents are frequently divisionals of larger pending applications which may issue with broader claims upon further prosecution. Hence even issued US patents may have additional claimed matter lurking behind an issued specification.

This is precisely what occurred with the Calgene *Brassica* transformation patent. The first patent issued had claims that encompassed any *Brassica* plant cell transformed by *Agrobacterium*. Competing organizations quickly considered alternative non-*Agrobacterium* based methods for transforming *Brassica* only to find out that a continuation in part was subsequently issued which had claims that encompassed all transformed *Brassica* cells, regardless of the method used to obtain them.

This second patent was not expected and caught a number of organizations off guard. Generally prevailing legal opinion did not anticipate this development since technically the processes of *Agrobacterium* transformation and mechanical (i.e. biolistics) transformation seemed to be distinct and a single patent that would encompass both technologies was considered unlikely, especially in view of the fact that the Calgene specification did not even exemplify the biolistic method. However, the USPTO essentially allowed that a transformed *Brassica* cell is a transformed *Brassica* cell regardless of the method of transformation and allowed the claim to any transformed *Brassica* cell produced by any means.

It is important to be cognizant of these possibilities when developing a freedom to operate strategy. It is very difficult to provide absolute assurance of the scope of a patent family since many applications are often left pending and in the United States additional matter can be added. Therefore any opinion on patent issues must be viewed in the "most likely" context rather than an "absolute" context. Although this may be a less-than-perfect situation, there is no true mechanism to position a freedom to operate strategy that does not contemplate the possibility of the issue of competing patents with broad claims even though the issue of a patent with a narrow scope appears more reasonable. These issues are generic to all biotechnology inventions and measures must be taken to ensure that the majority of the **most likely** intellectual property is avoided while acknowledging that some patents must be licensed. The present report examines the most likely roadblocks for the project at hand.

## Technical Considerations

The SLC technology provides a means to increase oil content in plant cells capable of accumulating oils. The practical interpretation of the technology suggests that major oilseed crops will benefit from the use of the technology by increased oil content and hence value. The current project is directed to the production of two industrial oils, erucic acid and a dienoic fatty acid using the SLC technology to provide the basis for increased oil content. The addition of a FAE 1 and DE5S gene to augment the SLC technology likely adds an inventive element to erucic acid production.

A number of issues are considered in detail within the scope of this report. These include not only practical aspects of reduction to practice and actual commercialization of the proposed lines, but the issues related to maintenance and development of intellectual property for this technology. In addition to these areas a discussion is included on the mechanisms that may be employed to developing a commercialization strategy for these particular products of the SLC technology.

In this regard there are commercialization issues that are not specific to this high erucic acid project, rather they are related to the practice of the SLC technology in general.

The actual technical demonstration of the SLC technology has been achieved and the results to date are extremely encouraging and well documented. Accordingly we are of the opinion that the technical practicality of the project is high and the workplan that has been submitted represents realistic goals. The outstanding credentials of the applicant team are recognized internationally therefore no additional comments on technical competency of the group are required.

The SLC technology will be discussed in greater detail in a following section, since it is relatively distinct from most of the existing oil modification technologies practiced to date. For project orientation a background examining existing oil modification technologies and the companies that control them is provided.

The area of plant oil modification has been dominated commercially by Calgene and more recently by DuPont although many other organizations have been active in this area. Most of the work carried out in plant oil modification has been aimed at increasing the fraction of a specific fatty acid within the lipid pool of a plant oil, such as high oleic acid, or development of a novel oil that is usually not found in a specific plant species such as the industrial oil canolas. The development of the high laurate canola by Calgene illustrates the latter.

The production of high erucic acid rapeseed has been of commercial interest for some time, although most of the increases in erucic acid content have been through conventional breeding. This project is somewhat confined in that it is focused on a

specific goal that is generally considered only to be commercially realistic with *Brassica* species. Therefore the Calgene *Brassica* transformation patent is a key road block to commercialization of the technology. The other issues for freedom to operate include component technologies that are germane to all plant biotechnology projects and the patents related to Fatty Acid Elongases (FAE 1) and Fatty Acid Desaturases (FAD, specifically the DE5S gene).

The specific intellectual property issues for these genes are described in the detailed analysis below.

Lastly, commentary on the proposed commercialization process and opportunities are provided. Although SWP and CanAmera are logical partners, we believe the potential difficulties in administration of the intellectual property component of the project have been somewhat underestimated. Difficulties that will be encountered in first developing the estate, second in the ownership of the technology and third gaining the required licenses to actually sell products are discussed.

The budget calls for $5000 per annum cost for legal fees which we assume will relate to patenting and licensing needs. This is an unrealistically modest figure considering that there are numerous licenses that are required to commercialize the product and that the current SLC patent application will be entering the International phase which will trigger costs associated with national phase prosecutions in countries where patent protection is needed. These additional costs will include translation fees, associate's fees, and maintenance fees. Although NRC will be providing some in-house legal expertise, the project is facing a number of important legal questions which are not likely to be covered by the $5000 per annum budget. Finally , there are issues for the development of the intellectual property for this particular project which relate to ownership and defense of the technology.

It is not anticipated that any of these first legal issues will be problematic, only that they will carry a cost which is likely to quickly exceed $5000. The costs to acquire the licenses for enabling technologies will consume that figure very quickly. Accordingly it is suggested that given the high probability of success of this project, an adjustment to the legal fees portion of the budget may be appropriate to ensure that the project is commercialized quickly. This cost may be borne by the industrial partners, however it is prudent to identify these concerns up front. Additionally there is the potential for problems in the partners sharing any intellectual property that arises from this project because joint ownership of patents often poses issues that are exceedingly difficult to resolve.

One of the biggest difficulties for the joint ownership of technology developed within the scope of this project is that it will be predicated on a patent already filed and assigned to the NRC. Development of a distinct patent family for the proposed technology in this project would be difficult as the published SLC application will be used as prior art and probably render the inventions in this project unpatentable. Hence

the continuation of the SLC technology in the US and through a World filing appears to be the logical route. However, this puts the NRC in the difficult position of having a portion of a patent estate which they already own now being owned by additional partners which may have a different view on the most important aspect of the technology.

Resolution of these issues may be very difficult and any arrangement may compromise the ability of the NRC to license the SLC technology to competitors of SWP or CanAmera. We believe the most logical way to proceed with the intellectual property would be to have the NRC retain ownership and SWP and CanAmera to have exclusive rights to the FAE 1 and DE5S aspects of this project technology with non-exclusive rights to the SLC technology. This provides protection for SWP and CanAmera to sell erucic acid varieties and allows the NRC to fully capitalize on the SLC technology. Furthermore it provides protection for PBI should CanAmera or SWP decide not to proceed. Should this happen PBI could then be free to commercialize the results with another partner, a situation which would not be possible if SWP and CanAmera retain ownership of the intellectual property.

Finally, there appears little in the way of commercial competition for this technology since it clearly works more efficiently that that already tested by various groups. Additionally the focus of this project on erucic acid levels clearly positions it outside of the main edible oil focus of most companies. The potential competition is discussed in more detail in the following sections.

## Enabling Technology For Oil Modification

Increases in the oil content of oilseed crops particularly canola has been the goal of plant breeders and molecular biologists for some time. The content of oil in canola seeds is inversely correlated with protein content such that increase in oil content are typically accompanied by decreases in protein. Generally it has been difficult to make significant gains in oil levels without sacrificing agronomic or performance characteristics required to license canola cultivars.

Many different strategies have been employed to increase seed oil content. For example, Murase *et al.*, describe the use of antisense RNA to inhibit storage protein biosynthesis and increase oil content (US 5,530,192, "Oilseed crops producing valuable seeds having altered amino acid composition and fatty acid composition). Ohlrogge *et al.*, attempted the overexpression of fatty acid biosynthesis enzymes such as acylCoA carboxylase (1997 Keystone Symposia on Cellular and Molecular Biology, Metabolic Engineering of Transgenic Plants, April, 1997). However none of these strategies have been particularly effective in increasing overall oil content.

The potential value of increased oil content of canola seed can be large. Currently the average yield of canola oil from a metric ton of grain is approximately 400 kilograms or 880 pounds of oil. This amount of oil is worth approximately $246 (US). An optimistic increase of 2.5% in oil content provides a premium of $6.16 (US) per ton of canola seed. Again, based on the volume of canola seed traded and the market share that a high oil canola cultivar can enjoy, the financial return can be considerable.

However, breeding efforts have reached a plateau for oil content and it is unlikely that a 2.5% gain in oil content, without the loss of the protein component can be accomplished by breeding alone. Therefore the application of recombinant DNA to overcome these limitations has been undertaken by a number of companies. As outlined below, numerous approaches have been forwarded and it is likely that this area will continue to be a strategic area for many organizations.

In terms of erucic acid content, breeding efforts have also reached a plateau and it appears that biotechnology approaches may be the only real alternative to achieving ultra high levels of erucic acid. Accordingly an overview of the technologies that have been suggested as a means to increase oil content or composition in general are provided.

Calgene, DuPont and other organizations have been actively developing molecular approaches to modified oil crops, although most of the technology is devoted to the modification of the oil profile as opposed to increased oil *per se*. However, high oil lines have been a primary goal of breeding efforts in most companies selling canola cultivars. Never-the-less, patents exist for high oleic acid canola derived via conventional or mutation strategies and individual patents for different ACPs and other

oil biosynthetic steps are numerous. **None of the patents however recite technology that encompasses the SLC technology**. Hence the SLC technology appears novel. However, as high oil is a goal of many organizations, the concept of high oil is recited as a goal of nearly every patent that describes technology aimed at oil modification.

To provide a frame of reference for the field of oil modification, the relevant patents related to modified oil canola have been assembled The following table illustrates the scope of the biotechnology patents that specifically relate to canola or oilseeds assigned to major players in the development of genetically altered seed.

**BRASSICA BIOTECHNOLOGY OR OIL MODIFICATION PATENTS, WORLD PATENT INDEX**

| Company Name | Patent Subject Matter (number of patents/number of countries) | Priority date, international scope of patent family jurisdictions |
|---|---|---|
| Calgene | 1) fatty acyl reductase (7/21) | 02/22/91, US, World |
| | 2) laurate, thioesterase (3/18) | 05/21/91, US, World |
| | 3) thioesterase (7/17) | 04/26/90, US, World |
| | 4) seed-specific promoters (2/2) | 8/8/91, US, EP |
| | 5) stearoyl thioesterase (1/20) | 09/29/95, US, World |
| | 6) desaturase (3/17) | 03/16/90, US, World |
| | 7) acyl carrier protein (10/19) | 07/28/87, US, World |
| | 8) wax esters (3/20) | 11/20/91, US, World |
| | 9) long chain (C24) fatty acids (1/20) | 11/30/93, US, World |

| | | |
|---|---|---|
| Cargill, Intermountain Canola or Cargill Semences or DuPont | 1) cultivar development (1/1) | 11/27/92, Hungary |
| | 2) oil processing (1/20) | 06/17/93, US, World |
| | 3)canola variety(2/20) | 04/27/93, US, World |
| | 4) canola variety (2/19) | 02/25/93, US, World |
| | 5) FA desaturase (3/23), FA desaturase (3/21) | 12/04/91, US, EP, World, 11/17/92, US, EP, World |
| | 6) ACP synthase II (3/20) | 11/15/91, US, World |
| | 7) Low linoleic canola (3/19) | 09/30/91, US, EP, World |
| | 8) High oleic canola (3/16) | 08/30/90, US, EP, World |
| | 9) ACP Thioesterase (3/18) | 12/20/91, US, EP, World |
| | 10) Steroyl ACP desaturase (7/18) | 05/25/90, US, EP, World |
| | 11 desaturases (1/71) | 12/14/95, US, World |
| ICI, Zeneca | 1) ACCase, fatty acid modification and use thereof for plastic synthesis (2/50) | 03/29/93, US, EP, Canada, Japan |
| | 2) Brassica napus ACCase gene and fragments, lower or modified oil content (3/39) | 11/28/91, US, EP, Canada, Japan, Australia |
| Pioneer Hi-Breds | 1) High oleic acid canola(1/1) | Canadian Patent Issued 02/07/95 (US Priority 12/31/87) |
| | 2) High oleic acid canola (1/13) | 12/31/87, EP 03/06/89, Australia, Finland, |
| | 3) High oleic acid canola (8/8) | Denmark, Japan, Norway, Britain, US |
| | 4) High oleic acid canola (5/14) | 12/31/87, EP |
| | 5) Fatty acid CoA desaturase (6/21) | 12/31/91, US, EP, Canada, Japan, Australia |

Not all patents related to oil modification are recited in the above table, there are also additional patents on similar technology, genes or approaches that are pending and are assigned to universities or public research institutions. However, the above table illustrates the complex field of intellectual property related to oil modification in oilseed crops. The particular WPI or US citations for these patents are appended.

From this selected analysis, it is clear that there are many different approaches that have been attempted for modifying plant oils. Some have been successful, while others have not proven to be suitable for commercialization. For the most part the simple addition or overexpression of acyl carrier proteins (ACPs) has not led to any dramatic increases in oil content.

Each of the patents recited above represent large patent families with various combinations of issued and pending applications and divisionals, etc. In general the claimed subject matter is not within the scope of the SLC technology. In fact the SLC technology arguably lies outside of all of these patents in terms of claimed subject matter since nearly all patents that relate to the use of novel acyltransferase activities rely on plant derived enzymes, not mutated fungal enzymes. This adds a considerable level of distinction to the SLC technology in terms of claimed subject matter. However, the use of novel acyltransferases to modify oil in oilseeds is not new and the SLC technology will face the inevitable prior art arguments and rejections that will need to be overcome. This is expected for any patent application in plant biotechnology.

Calgene is a leader in the field of oil modification, and has developed a prodigious patent estate.. They have issued patents which cover many novel genes in the oil biosynthetic pathway with an emphasis on industrial oils outside of the very long chain fatty acids. The patents that Calgene has obtained include lysophosphatidic acid acyltransferases (LPATs), but claims are limited to LPATs with specificity towards medium chain fatty acids, not long chain fatty acids. Accordingly the SLC technology is not anticipated by the Calgene acyltransferase patents.

Overall, Calgene's patent estate on oil modification is heavily skewed towards the production of medium and short chain fatty acids with little emphasis or claimed subject matter on very long chain fatty acids. Hence, outside of the patents related to seed specific promoters and *Brassica* transformation, the issued Calgene patents on oil modification do not pose a block for the current project. The seed specific promoter patents really only affect the promoter choice for this project, at least from a North American perspective.

The Calgene patents (e.g. US 5420034) related to seed specific transcription for modifying oil production claim a *Brassica* plant comprising:

*a DNA construct comprising, in the 5' to 3' direction of transcription,*

*a transcriptional initiation region from a gene that encodes a product preferentially expressed in a plant seed cell as compared to other plants cells,*

*a DNA sequence of interest other than the native coding sequence of said gene, and a transcriptional termination region,*

*wherein said gene is a napin gene an acyl carrier protein gene or an EA9 gene.*

Accordingly the use of the napin promoter, a promoter from an acyl carrier protein or the Calgene "EA9" gene is controlled by this patent and we notice that the present project contemplates the use of the napin promoter (page 2, under "constructs"). We believe that napin should be avoided in a commercial product and the Kti3 promoter mentioned in the project, if it has a similar pattern of expression could be substituted. Alternatively PBI may have a novel seed specific promoter that may be employed within the scope of the project and be free of issued intellectual property.

Similarly the use of the 35S promoter should be avoided since Monsanto controls that promoter and a combination of napin, 35S promoter and *Brassica* transformation makes this project very much at the mercy of Monsanto in terms of freedom to operate. Obviously some of these roadblocks can not be avoided, however, it is better to avoid as many as possible. The Calgene/Monsanto situation is discussed in further detail in the following section.

We consider Cargill, Intermountain Canola and DuPont together since there has been a relationship in the past between the parties. Cargill and Intermountain Canola combined do not have a particularly large IP estate for oilseeds in the absence of the DuPont patents. It is not surprising that few biotechnology patents appear assigned to Cargill. The acquisition of Intermountain Canola would not lead to any change in assignment on the World Patent Index (WPI) and thus it is unclear that Intermountain Canola had any technology actually assigned to it before the takeover. Certainly no assignment to Intermountain Canola or variants of that name were seen on the WPI data base. However a new patent assigned to Cargill has appeared related to desaturases. This patent (WO 9721340) claims a priority of 12/14/95 and is related to a delta 12 and delta 15 desaturase gene from *Brassica* or sunflower. These particular desaturase activities are not part of the planned enzyme activity for the present project.

It is likely that Du Pont controlled all the biotechnology that may have been part of Intermountain Canola efforts. It is presumed that the rights to the patents related to canola *per se* stayed with Intermountain Canola (and now Cargill), biotechnology related to oilseeds in general stayed with DuPont.

Examination of the Du Pont patents reveals modified edible oils to be the primary target. Both conventionally bred canolas and genetically modified canolas are be considered likely commercial objectives. There are a number of Du Pont patents by Fan,

DeBonte and Loh that describe various canola types (conventionally bred) with altered oil, there is a high oleic acid (71%) canola patent which was filed in the US in 1990, but has not been issued yet. Given our experience with US patent prosecution, one would have expected the patent to issue however, a US CiP dated 1991 is also listed as a priority document. It is assumed therefore that the file is still active. DuPont also has a number of patents related to FA biosynthesis and modification genes which could be useful for canola modification. However, none of these pending applications relate to erucic acid.

ICI (and Zeneca) have developed a complicated intellectual property estate that has significant potential to compete in the modified oils arena. Included in those patents where *Brassica* was identified as a primary target is a series of patents related to the synthesis of polyhydroxyalkanoate (PHA) under the control of a chemical switch that allows the production of PHA to be regulated by application of an inducer. In the absence of an inducer PHA is not made. The chemical switch they have identified and shown to function in plants is a alcohol-inducible genetic systems isolated from the fungi *Aspergillis* modified to work in plant cells. PHA can be made into polyhydroxybutyrate, a biodegradable plastic.

The source of material for the synthesis of PHA is Acetyl-CoA in the seed. They have also cloned a gene for Acetyl CoA carboxylase (ACCase), a gene central to oil biosynthesis. The gene was isolated from *B. napus* seeds. The potential use of this gene is for down regulating ACCase, making more Acetyl CoA available for PHA synthesis, or, upregulating the gene to make more oil. Additionally the gene can be used to confer resistance to the aryloxyphenoxy-proprionate and alkylketone herbicides in cereal crops.

There is also a patent on maize Acetyl CoA carboxylase filed (3 members of the patent family, filed in 39 countries) most recently in late 1994. This patent was filed before the *B. napus* patent and was probably a result of early work carried out in maize. Potentially the initial cloning efforts in maize made the *B. napus* cloning possible. However, the two patent families are distinct.

Various combinations and permutations of these genes and gene fragments can be contemplated, essentially the ultimate goal of this technology seems to be the development of oilseed crops that can either make more specialty oils or be switched to make plastic precursors. Interestingly, one of the schemes proposed for the synthesis of plastics is to use the chemical switch to control the synthesis of PHA during germination of oilseeds. The idea is that one could "malt" oilseeds as is done now for barley, but instead of recovering malt, one would recover either PHA or polymerized PHA (polyhydroxybutyrate). We did not find any information in the ICI patent family which would suggest that erucic acid is a primary target for their efforts.

Pioneer Hi-Breds has purchased a number of patents related to *Brassica* through the acquisition of Allelix and Paladin Hybrids Inc. Of possible relevance to the direction of the oil modification technology of Pioneer is the issued Canadian patent on high oleic acid canola originally filed in 1988. It was issued in Canada on February 07, 1995. The

primary claim of this patent relates to a plant that has 80% oleic acid content in the oil fraction. The means to produce this plant comprises the use of a mutagen followed by selection and screening. The original priority document is a US filing made in 1987. From this priority document a complex family has developed including various European patents. There are similar patents related to mutagenesis for developing altered oil and herbicide tolerance. A US patent was issued February 02, 1995 (5387758) that includes both altering oil and imparting herbicide tolerance by mutagenesis. It is possible that mutagenesis may be employed for erucic acid varieties, but we do not believe PHI has a significant interest in erucic acid varieties.

PHI has apparently licensed the patent family for the Delta-9 desaturase from a group in Kentucky. The enzyme has been isolated from rat and introduced into plants. A primary concept of this approach is that at higher temperatures the rat enzyme will still function efficiently when compared to a plant enzyme, thus environmental effects on levels of unsaturated fatty acids will be minimized. Cold tolerance (through increased levels of unsaturated fatty acids) is also claimed. It appears that PHI is focusing on edible oils.

It is of interest to speculate on the scope of the oil modification technology contained within the recently announced PHI/DuPont venture "Optima Seeds". It is presumed that the investment by DuPont in PHI allows for access to PHI technology related to oil modification and vice versa. Hence the leader in the area of modified oil in terms of technology and potential markets may now be the PHI/DuPont joint venture.

**In summary, none of the major companies active in oil modification via biotechnology have developed a competitive technology related to erucic acid production that would impact this project.**

However, some of the pending patents will be considered prior art against the SLC technology since a similar general concept is disclosed. Moreover, none of the pending or issued patents appear to have claims which block the practice of the SLC and erucic acid technology at a conceptual level.

## Intellectual Property Related to Erucic Acid

The intellectual property situation for conventional high erucic acid *Brassica* is relatively straightforward since the high erucic acid trait is essentially in the public domain. Accordingly there is no known patent that governs the genetic trait of high erucic acid content in the genus *Brassica*. Searches of the US and WPI databases from 1971 to 1997 confirm this. Therefore the germplasm in which the invention will be practiced is publicly available.

The modification of this germplasm by the introduction of a recombinant DNA is not without significant intellectual property concerns. As discussed, above the development of transgenic *Brassica* is dominated by the issued patents (US 5188958 and US 5463174, Moloney et al, assigned to Calgene - both citations appended). Monsanto now controls all of Calgene intellectual property.

The Calgene patent US 5188958, issued February 23, 1993 was filed August 14, 1989 and was a continuation-in-part of a US application filed May 26, 1987 which is a continuation-in-part of a priority application filed May 29, 1986. Both priority applications are now abandoned. The issued patent has 9 claims. US 546317, filed September 11, 1992, issued October 31, 1995 is the same specification as US 5188958 and has similar claims with the exception of scope.

The first patent US 5188958 claims all transformed *Brassica* plants, cells and cultures derived by using *Agrobacterium* and the method set forth. Of particular interest is the first claim which recites:

*A transgenic Brassica species cells and progeny thereof comprising an expression cassette wherein said cells are characterized as onco-gene free and capable of regeneration to morphologically normal whole plants, and wherein said expression cassette comprises, in the 5' to 3' direction of transcription:*

*(1) a transcription initiation region functional in Brassica cells;*
*(2) a DNA sequence comprising an open reading frame having an initiation codon at its 5' terminus or a nucleic acid sequence complimentary to an endogenous transcription product which when expressed will alter the phenotype of said transgenic cells;*
*(3) a transcription termination region functional in Brassica species cells;*
*(4) a right border of T-DNA; and*
*(5) a structural gene capable of expression in said Brassica providing for selection of transgenic Brassica species cells;*
*wherein said expression cassette is capable of altering the phenotype of said Brassica species cells when said cells are grown under conditions whereby said DNA sequence or said nucleic acid is expressed.*

This claim encompasses all *Brassica* plants transformed by *Agrobacterium*. This claim does not dominate non-*Agrobacterium* produced transgenics. However, Calgene

was able to increase that coverage in a continuation in part that was allowed with broader claims. US 5463174 issued subsequently to the first Calgene *Brassica* transformation patent has a very similar broad first claim which reads:

*A transgenic Brassica species cells and progeny thereof comprising an expression cassette wherein said cells are characterized as onco-gene free and capable of regeneration to morphologically normal whole plants, and wherein said expression cassette comprises, in the 5' to 3' direction of transcription:*

*(1) a transcription initiation region functional in Brassica cells;*
*(2) a DNA sequence comprising an open reading frame having an initiation codon at its 5' terminus or a nucleic acid sequence complimentary to an endogenous transcription product and;*
*(3) a transcription termination region functional in Brassica species cells;*
*wherein at least one of said transcription initiation region and transcription termination region is not naturally associated with said DNA sequence or said nucleic acid sequence; and wherein said expression cassette imparts a detectable trait when Brassica species cells are grown under conditions whereby said DNA sequence or said nucleic acid sequence is expressed.*

This claim encompasses any *Brassica* cell that has an introduced "modified" gene Modified is defined as containing a non-natural promoter or terminator sequence. Given the fact that the SLC gene is derived from yeast and the native yeast promoter is replaced with a plant promoter, the SLC technology in *Brassica* is dominated by this patent claim.

- **The Calgene transformation patent is a primary roadblock to commercialization and a license will be required.**

In addition to the Calgene patents consideration must be given to the fact that the current project is dependent on technology (the yeast SLC1 gene) controlled by the University of Kentucky. Given the fact that the prosecution of any and all intellectual property related to the yeast SLC1 gene is controlled by the University of Kentucky and the SLC invention of MacKenzie *et al* is predicated on the use of this particular gene, freedom to operate or more importantly restrict the activities of others and gain a dominant position in this area will require success of both groups.

Accordingly the protection of the MacKenzie *et al* SLC technology will be to a large extent influenced by the scope of protection gained by the University of Kentucky. It is not clear if a related gene (such as other fungal acyl transferases) can be utilized within the scope of the MacKenzie *et al* SLC technology to the same success, but in a worst case scenario it is possible to envision competitive technologies being practiced outside of the scope of narrow patents on the yeast SLC1 gene. However, it appears that the utility of the SLC technology is highly dependent on the particular SLC gene and therefore the current patent position of the SLC technology appears very promising.

The Mac Kenzie SLC specification has been reviewed and the scope of potential protection for the proposed high erucic *Brassica* product is within the specification as written or would be possible within a continuation in part.

- **We are of the opinion that the current SLC filing covers the intended products of this project and believe that adequate protection for the high erucic acid trait as produced by the SLC technology will be afforded by the present patent family or a continuation in part.**

However, it must be mentioned that the issue related to a license for transformed *Brassica* must be resolved for the commercialization (i.e. sale and entry of the product into commerce) of the products to take place.

In terms of genetically engineering high erucic acid content in *Brassica*, two patents related to acyltransferases from plants were identified as potentially damaging to this project. WO 9609394, Frentzen *et al*, assigned to KWS and Slabas *et al*, WO 9413814, assigned to Nickersons. Both patents relate to acyltransferases from plants and the use thereof to increase or alter fatty acid content in plants, with particular reference to erucic acid.

However, WO 9609394 recites an acyltransferase from a vegetable source and provides a DNA sequence of such an enzyme. It has claims to genes with varying degrees of homology to their claimed sequence. The homology ranges from 35% or more, but claims to homologous sequences are always difficult to support and enforce. It is likely, based on the work of Mac Kenzie, that the yeast SLC gene is not highly homologous to a plant enzyme and would not be encompassed by the current proposed claims. This is likely to be particularly true at the DNA level due to the differences of the codon preference between yeast and plants.

WO 9413814 recites an acyltransferase with specificity towards the 2 position of the glycerol backbone and in particular specificity towards adding erucic acid to this position. This is similar in focus to the SLC technology but differs from it in many ways including the bias towards "insoluble" acyltransferases. The claims of the patent recite a recombinant or isolated DNA sequence that hybridizes to their described sequence under "stringent" conditions. The acyltransferase exemplified in the specification was derived from maize and the maize cDNA was used to isolate a *Brassica* acyltransferase. As above, the claims recite homology and are directed towards plant acyltransferases. Accordingly we believe that the SLC technology would not be encompassed by the claims.

Although WO 9413814 and WO 9609394 both recite acyltransferases and the use of these genes to either increase oil or specifically modify oil, the claims are directed to plant derived acyltransferases or acyltransferases highly homologous to the claimed plant derived genes.

**• It is believed the SLC technology, which relies on a mutant yeast gene would lie outside of the scope of these patents.**

The actual level of homology between the SLC gene used in this project and the acyltransferases claimed in WO 9413814 and WO 9609394 needs to be evaluated at the DNA and protein level to ensure that the SLC gene is outside of the claimed subject matter. We presume the researchers have this information and based on the preprint of the paper we received from PBI, the homology to meadowfoam or coconut acyltransferases is only 24%. As these are representative plant acyltransferases it is expected that the SLC gene is not within the scope of the claimed homology of WO 9413814 and WO 9609394.

The project is also dependent on the activities of two additional genes, the FAE 1 from *Arabidopsis* and the DE5S from meadowfoam.

Searches that were conducted for published patents related to FAE 1 genes failed to detect any citations on the US or World patent databases. There are two possible explanations for this. First, it is possible that there are no patents that have been filed on FAE 1 genes. The second possibility is a pending application that has been filed but not published. As the FAE 1 gene from *Arabidopsis* was cloned by the group at DNAP is likely to be the subject of a pending patent. However, this gene was cloned in 1995 and the paper published in March, 1995 thus the publication of an application would have been expected before now. It is possible that there is only a US patent pending and as information on pending US patents is not published, but this would be unusual as the usual practice would be to file a World Patent application on the gene. It may be that they did not see value or that there was no commercial outlet for their potential targets.

**• The FAE gene (or more accurately the FAE1 gene) that would be utilized as part of this project has been published and at present it appears there are no issued or pending patents that cover the use of the gene.**

However, this alone does not preclude the possibility of a patent eventually appearing that would govern the gene. Given the fact that the gene has been published for some time we believe that a broad patent on the gene has not been filed by DNAP or any of the researchers at present.

Similarly the delta 5 desaturase gene from meadowfoam has not yet been patented or any filed patent has not yet been published. The US and WPI databases were searched for a delta 5 desaturase and no citations for such a enzyme activity were discovered. Although there are many other desaturase activities that are the subject of various patents, all of the desaturases are mainly claimed by DNA and protein sequence homologies or specific activities.

The most comprehensive desaturase patent in terms of claimed subject matter is WO9411516, Lightner *et al*, assigned to DuPont. This application was filed October 15, 1993, claiming a priority of November 17, 1992. This application details the isolation of a delta 12 desaturase and has claims to any desaturase with homology to the exemplified sequence. However, the specification does not contemplate the isolation of a delta 5 activity. It does suggest that all desaturases will have a high degree of homology and hence the exemplifies DNA could be used to isolate any number of desaturases.

The claim strategy of this patent application relates to amino acid identity rather than homology which is a less defined term. We can not provide any comment on the homology of the delta 5 desaturase contemplated within this project since the gene has not been isolated yet, but if it has an amino acid identity of greater than 50% to the published sequence in the application it could be encompassed by the claims as written. However, it is not very likely that the gene from meadowfoam will be 50% identical to a soybean or canola delta 12 desaturase. This can only be verified once the gene is isolated. This patent is appended for future reference and amino acid comparison to the gene once it is isolated by the project group.

- **The delta 5 desaturase activity is not recited in published patent applications and unless it is highly homologous to published desaturases it will not be encompassed by the pending or currently issued claims related to desaturases.**

It is somewhat surprising that patents or applications related to the FAE 1 or (to a lessor extent) the DE5S genes were not identified. Usually genes which are detailed in research articles published from companies are the subject of previously filed patents. The fact that citations to the FAE 1 gene were not apparent argues that the gene is within the public domain.

A brief note on the patenting process allows for this logic to be followed. Generally a discovery is protected by first filing a priority document which describes the technology. Usually a second application, which is more complete and expanded in scope is filed 12 months after the initial filing. This second filing is then published as a World or European application 18 months after the filing date of the initial application. Hence any research publication is often followed within 18 months by a published World or EP application.

In the case of the FAE1 gene, the possibility of a patent pending on this gene is quite remote given the fact that the article was published over two years ago. In the case of the DE5S gene, it has not been isolated so there can be no corresponding patent application.

- **The FAE 1 gene and the DE5S genes do not appear to be the subject of existing patents and thus will be available for use in this project without the requirement for licensing.**

Accordingly the main intellectual property concern for this project is the *Brassica* transformation patent and the component technologies used in construction of the vectors. These have been detailed in a previous report to AFIF, but will be reviewed here as part of the intellectual property checklist for this project. Included in this list are the following component technologies:

**General recombinant DNA methods** - controlled by Stanford with the Cohen & Boyer patents, available for license under reasonable terms and conditions.

**DNA components** - transformation vectors, promoters, etc., are for the most part controlled by participating groups or freely available. it is suggested that the group avoid patented components as much as possible.

**Transformation methodology** - Obviously the biggest issue at present is the transformation patent of Calgene controlled by Monsanto. SWP is likely to be the partner that can gain this license and they probably have the ability to gain this license in exchange for certain arrangements with Calgene. However, it is not know if the relationship between Calgene and SWP extends to these rights. Since SWP knows Calgene has control of the transformation technology and SWP is proceeding with this project, an arrangement may have already been arranged or contemplated. Although commercialization of this technology is important, it is up to the commercial partners, in this case SWP or CanAmera to ensure that the licenses are in place, since they will be selling the seed.

Additionally the transformation system patents for the use of binary or co-integrate vectors are important. It is expected that a the license for either the binary or cointegrate system can be obtained at a cost in the few hundred thousand dollar range and will be needed by the commercial partners in order to sell seed or derived products.

Outside of these issues, the intellectual property situation for this project is relatively clear. The technology itself is controlled by one of the participants, the additional genes that will be employed within the scope of the project are either freely available or will be isolated as part of the project and therefore may be eligible for patent protection. The primary issue of freedom to operate with transgenic *Brassica* and transformation vectors needs to be resolved by the commercial entities which will be responsible for sales of genetically altered seed.

## Discussion and Conclusions

The present project is dominated by the same intellectual property roadblocks that all developers of transgenic *Brassicas* face, namely the transformation patent controlled by Calgene and the transformation systems patents controlling the use of *Agrobacterium*

***These roadblocks can not be overcome without one of the commercial partners obtaining a license for the transformation technology.***

The opportunity for SWP or CanAmera to obtain a license does not appear to be restricted due to any competitive interests of Calgene or Monsanto. Moreover, we suspect that the use of the SLC technology by Calgene in their modified oil program would be of great interest and thus may possibly be used as a bargaining tool in license negotiations. In consideration of the fact that SWP or CanAmera will be the parties actually selling products it is up to SWP or CanAmera to obtain rights to these technologies. Although it may also be useful for the NRC to obtain a license the negotiation of an agreement that would allow the transfer of rights to third parties such as SWP and CanAmera is an unrealistic expectation.

In addition to potential roadblocks caused by intellectual property held by others there are additional considerations relating to the ownership of patents that may result from the project itself.. The current plan of joint ownership could lead to conflicts at a future time during the commercialization of the technology. The crafting of an agreement for joint ownership of the resultant intellectual property that protects the interests of all parties may be difficult achieve thus the sole ownership of the technology by the developing party in this project may offer a better way to proceed.

An argument against the joint ownership of the technology relates the difficulties discussed earlier regarding joint ownership of intellectual property in general and the possibility of placing NRC in the position to have to bargain with Calgene for rights to transformation by trading SLC technology. Since the NRC does not sell canola seed, there is no compelling reason for NRC to obtain a transformation license for participation this project as this is the responsibility of the commercial partners. If project technology is jointly owned by SWP, CanAmera and NRC, and has elements of the SLC technology that are based on continuations of the SLC technology now held by the NRC the potential for conflict or interest arises. Clarification of future intentions concerning the anticipated intellectual property rights that will result from the program would be advisable.

The current patent governing the use of the SLC technology is assigned to NRC and encompasses the use of the SLC gene in oilseed crops. However, this project also will incorporate the FAE 1 and DE5S gene into SLC lines. This becomes somewhat

problematic if the technology developed as part of this project is incorporated as a continuation of the already filed SLC technology since there will be a complex situation regarding rights and costs associated with the prosecution of specific claims. Not only that, but should SWP and CanAmera own a portion of this technology, they may be more inclined than the NRC to trade rights to third parties in order to reduce any potential license fees. The SLC technology is potentially a very valuable technology and is wholly owned by the NRC. Joint ownership of a continuation of the technology implies certain rights to the owners. The goals of NRC, SWP and CanAmera will differ considerably and ensuring that NRC protects the SLC technology for licensing to other parties will not be a common goal of SWP and CanAmera.

***Consideration should be given to NRC sole ownership of the technology with the granting of exclusivity to SWP and CanAmera for the specific products contemplated in this project while retaining rights for the SLC technology for other clients and uses.***

Such an arrangement provides complete exclusivity for SWP and CanAmera for production of erucic acid varieties, and places the burden of obtaining a transformation license on the actual organization selling seed. This also allows the commercial partners to negotiate for a license to cover exactly what is derived from the project as opposed to a more general license for transformation. Such an arrangement may be less expensive and easier to negotiate than for example the NRC obtaining a transformation license and sublicense to SWP or CanAmera. In addition, such an arrangement allows NRC to continue to direct patent prosecution and patent estate development which is important to their mandate.

Alternatively, should this technology emanating from this project be patented as a new application jointly owned, the existing SLC application will be cited as prior art and it will be a very difficult obstacle to overcome. Although such an approach allows for a clear delineation of the scope of the shared technology, the likelihood of success is much reduced. Assignment of a divisional application or assignment to specific claims of a continuation is possible, however it could involve a considerable amount of time to fully negotiate these rights between the parties. Certainly the NRC will wish to retain complete control over the SLC technology given its apparent wide utility while SWP and CanAmera will want to control the FAE 1 and DE5S aspects of the technology. This becomes exceedingly complicated when divided in this fashion.

***Any new intellectual property developed as a result of this project may be difficult to administer if the patents are jointly owned by all parties.***

It is not impossible, but it will take creative thinking to preserve the rights to a product that incorporates many related technologies. It appears that the simple solution to this potential problem is for NRC to retain ownership and provide exclusive rights to the partners.

The ability to commercialize products of this research appear only to be limited by the *Brassica* transformation patent. Current pending and issued patents related to oil modification in plants do not have claims that encompass the SLC technology nor are there any public patent applications that claim the DE5S or FAE 1 gene. Hence the only identifiable intellectual property issue for the present project, outside of specific component technologies that are easily avoided, is the *Brassica* transformation patent.

At present there is no means to escape the dominance of the *Brassica* transformation patent. The commercial partners in this project will need to obtain a license for that technology in order to commercialize the varieties produced as a result of the PBI research efforts.

The potential value of unltra-high erucic acid canola will likely support the licenses for transformation and *Agrobacterium* since it appears that the potential market for such products is substantial. However, SWP and CanAmera will ultimately be responsible for a license and payment of royalties. Hence their interest in the project underscores the potential return even with the consideration of licensing needs. Given the level of investment thus far, the potential market for the product and the exclusivity that will be enjoyed by SWP and CamAmera, we are of the opinion that there will be sufficient economic return in this product to support the license for both transformation and *Agrobacterium* technologies.

Accordingly we believe this particular project has a good potential for success both at the scientific and commercial level and the unavoidable license fees associated with the enabling technology need to practice the invention will not consume the majority of the potential economic value.